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VOLUME 31 APRIL-MAY, 1953 NUMBERS 4 and 5

# PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

## XXXVII. PILOT PLANT FERMENTATION OF BEET MOLASSES—DESCRIPTION OF PLANT AND RESULTS[1]

BY J. A. WHEAT

ABSTRACT

A pilot plant capable of handling 880-gal. batches of beet molasses mash is described and operational procedures given. Mash was sterilized in a continuous cooker and fermented in a 1500 gal. stainless steel vessel provided with cooling coils, means of aeration, foam breaker, and automatic temperature and pH control. Yields of 143–187 lb. of 2,3-butanediol per 1000 lb. of sugar-beet molasses were obtained by fermentation with *Aerobacter aerogenes* and *Pseudomonas hydrophila*. About 98% of the sugar was converted and, on the basis of total products, fermentation efficiency varied from 77 to 98%.

## INTRODUCTION

In 1950 Canada produced over a million tons of sugar beets which yielded as a by-product about 40,000 tons of molasses (14). Of the total production, about 35% was grown in Ontario, 35% in Alberta, 20% in Manitoba, and 10% in Quebec (15). The composition of beet molasses is given in detail by McGinnis (5). Briefly, beet molasses contains about 52% sucrose with a trace of reducing sugar and about 31% other solids. Present uses (5) include animal feed and production of citric acid, alcohol, and yeast by fermentation. Since the use of beet molasses for the 2,3-butanediol fermentation would provide another potential outlet for the raw material, this fermentation was undertaken on a pilot plant scale. Pilot plant production of *levo*-2,3-butanediol from whole wheat and barley mashes by *Bacillus polymyxa* has been described previously (2, 16).

Three organisms, *Bacillus polymyxa* (12), *Aerobacter aerogenes* (3), and *Pseudomonas hydrophila* (1, 7), have been reported to give good yields of butanediol from beet molasses on a laboratory scale. Since preliminary pilot plant trials of *B. polymyxa* were unsuccessful, *A. aerogenes* and *P. hydrophila* were chosen for the present work. *A. aerogenes* produces a mixture of the *meso*, *racemic*, and *dextro* isomers of 2,3-butanediol (19) and *P. hydrophila* produces a mixture of *meso*, *racemic*, and *levo* isomers (7). *B. polymyxa*, on the other hand, produces essentially a pure *levo* isomer (8).

[1] *Manuscript received December 8, 1952.*
*Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Ont. Issued as Paper No. 150 on Uses of Plant Products and as N.R.C. No. 2956.*

Laboratory studies (6) have indicated that beet molasses is deficient in available phosphorus. Triple superphosphate was added for *A. aerogenes* fermentations and dibasic ammonium phosphate for *P. hydrophila*. Laboratory tests (1) indicated that triple superphosphate was not suitable for *P. hydrophila* fermentations. In contrast to earlier laboratory findings (1), dibasic ammonium phosphate has given higher yields than corn steep liquor in larger scale *P. hydrophila* fermentations. Hence, corn steep liquor has not been used in the present investigation.

This paper describes pilot plant fermentations of beet molasses in 880-gal. batches in a 1500 gal. stainless steel fermenter after sterilization in a continuous cooker. The recovery of butanediol from fermented molasses mashes will be described in a later paper (21).

## LABORATORY METHODS

Inocula were prepared by transferring cultures of *Pseudomonas hydrophila* N.R.C. 492 (7) or *Aerobacter aerogenes* M148 (7) from 8- or 24-hr. molasses–agar slants to 4-liter bottles containing 2 liters of a fortified molasses medium. Inoculum media for *A. aerogenes* fermentations contained 5% molasses and 0.5% corn steep liquor. For *P. hydrophila* fermentations, the inoculum media contained 10% molasses and either 0.5% yeast extract or 0.005% phosphorus pentoxide added as dibasic ammonium phosphate (1). The bottles were incubated for 16 or 24 hr. at 32–34° C. on a rotary shaker operated at 100 r.p.m.

The analytical methods reported by Neish (9) were used, with some modification, for diol, ethanol, acetoin, and sugar in fermented mashes. Diol plus acetoin was determined by the periodate oxidation method; acetoin was determined separately and diol obtained by difference. Ethanol results were not corrected for the diol and acetoin that distilled over with the ethanol. Sugar content of the molasses was determined by the Lane-Eynon method (13). Although most of the sugar in molasses is present as sucrose, it is more convenient to express it as invert sugar and this has been done throughout the paper.

Sugar fermented was calculated from the weight of the sugar in the original molasses and from the weight of the sugar present after the fermentation. Fermentation efficiency was calculated by dividing the sum of the weights of diol, acetoin, and ethanol by half the weight of invert sugar in the molasses. Efficiency is based on half the weight of the sugar because half is converted to carbon dioxide (4). Yields were expressed as lb. per 1000 lb. of molasses corrected to the basis of a molasses containing 50% invert sugar.

## PILOT PLANT EQUIPMENT

Fig. 1 is a flow sheet showing the main units of the equipment and most of the control instruments.

### *Mash Preparation Equipment*

The continuous sterilizing system was essentially the same as that recommended for cereal grains by Unger, Willkie, and Blankmeyer (17), but of

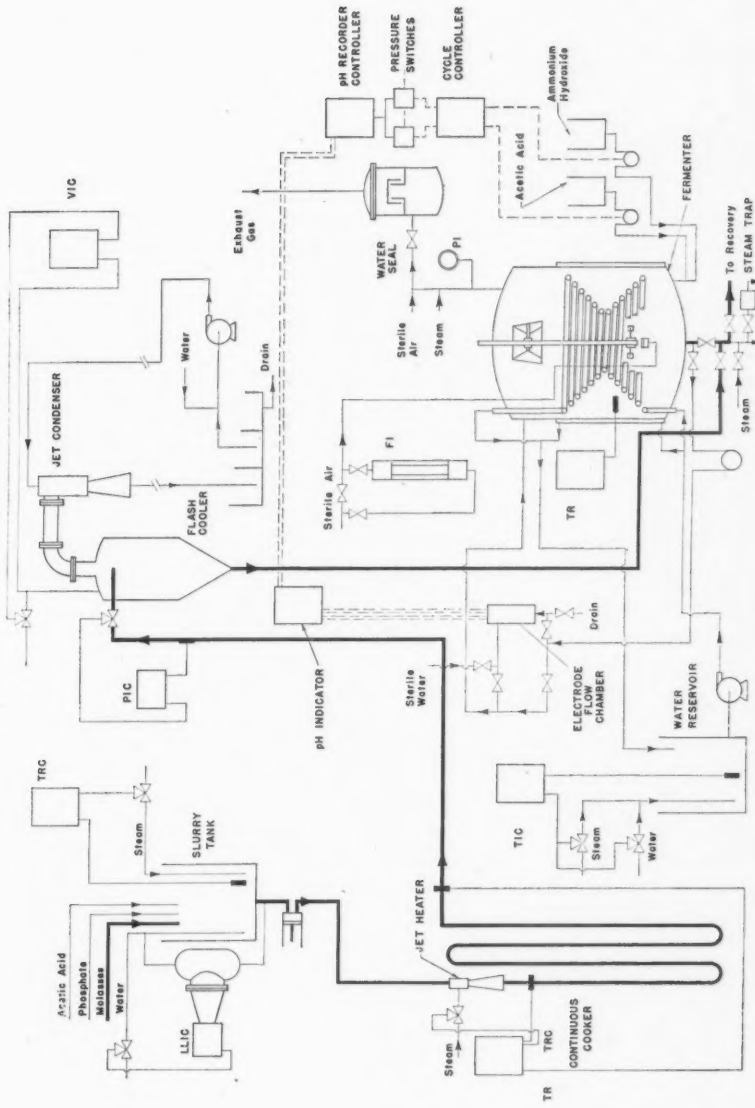



FIG. 1. Flow sheet for fermenter and continuous cooker. T = temperature, P = pressure, F = flow, LL = liquid level, I = indicator, R = recorder, C = controller.

smaller capacity. Molasses flowed by gravity from a concrete storage tank to two weigh tanks with a capacity of about 500 lb. each and was then pumped to a 50 gal. slurry tank. The storage tank, weigh tanks, and transfer pump are not shown in Fig. 1. Mash was heated in the slurry tank by direct steam sparge regulated by a temperature controller and a constant liquid level was maintained by automatically controlling the water supply.

Raw mash was pumped to the continuous cooker with a duplex proportioning pump, which had a total capacity of 350 gal. per hr. and which controlled the over-all processing rate of the continuous cooker. The cooker itself consisted of 150 ft. of 1 in. monel pipe insulated with standard thickness magnesia pipe insulation. Mash was heated by mixture with steam (200 p.s.i.) in a jet heater* made of Hastelloy. The inlet temperature was controlled by regulating the steam supply and the temperature near the end of the cooker was recorded on the same chart. To prevent mash from boiling, the pressure in the cooker was maintained above the vapor pressure by a control valve placed as close as possible to a flash cooler.

The flash cooler consisted of a monel cylinder 2 ft. in diameter and 2 ft. high with a 60° conical bottom and a tangential mash inlet. Vapor from the cooler was condensed in a jet eductor–condenser from which water was discharged through a barometric leg to a well. A portion of the water from the well was combined with make-up water and recirculated to the condenser with a centrifugal pump. A constant vacuum was maintained in the flash cooler by automatic regulation of an air bleed. A ball float operated a pneumatic transmitter so that the liquid level in the cooler could be observed at the control panel. (The liquid level instruments are not shown in Fig. 1.) Cooled mash flowed by gravity from the flash cooler to the fermenter through a $1\frac{1}{2}$ in. monel pipe.

*Fermenter*

The fermenter, which was fabricated of type 304 stainless steel, was 6.5 ft. in diameter and 6.5 ft. high on the straight sides with standard dished head and bottom giving a total volume of about 1500 gal. It was provided with a baffled jacket $\frac{1}{2}$ in. deep and contained a coil of 385 ft. of $\frac{3}{4}$ in. tubing as in Fig. 1. Two 4-in. windows for observing the mash level or foaming conditions were placed in the top of the fermenter. Mixing was provided by a vaned disk agitator 11 in. in diameter driven at 420 r.p.m. by a 5 h.p. motor. Swirl was prevented by four baffles 8 in. by 36 in. placed inside the coil. A mechanical foam breaker in the form of a truncated cone 9 in. high with a bottom diameter of 18 in. and a top diameter of 15 in. was placed on the agitator shaft about 3 ft. above the liquid level. Inside the cone were four vanes shaped as shown in Fig. 1. Air was forced into the fermenter through a 2 in. diameter disk of porous stainless steel (mean pore opening of 0.0025 in.) placed just below the center of the agitator. Rate of air flow was measured with a rotameter. Exhaust gas passed through a water seal consisting of a single 4-in. bubble cap in a

* *Schutte and Koerting, Philadelphia, Pa. Fig. 320, size 1 in.*

vessel 9 in. in diameter by 12 in. high. Samples were taken by closing off the exhaust gas vent, increasing the pressure in the fermenter to about 3 p.s.i., and removing the sample by means of a pipe through the top of the fermenter.

*Temperature Control*

To control the temperature of mash in the fermenter, water was circulated from a 50-gal. copper reservoir through the coil and jacket. The temperature of the water was controlled by an indicating instrument that regulated the addition of steam or cold water. A separate instrument was used to record the temperature of the mash in the fermenter.

*Measurement and Control of pH*

Measurements of pH were obtained either with electrodes inserted in a flow chamber through which mash was circulated or with electrodes immersed directly in the mash. For the flow method, mash was circulated with a rotary pump from the bottom of the fermenter, as shown in Fig. 1, through the flow chamber and back to the top of the fermenter. For the immersion method, steam-sterilized high temperature electrodes* were inserted through the top of the fermenter and immersed well below the mash level.

The output air pressure of the pH recorder controller (proportional response only) actuated two mercury switches with adjustable cut-in and cut-out points (Fig. 1). The electrical output of these switches was sent through two more mercury switches on a time cycle controller to two motors driving sliding vane pumps.† These pumps added 80% acetic acid or 26° ammonium hydroxide to the mash at a rate of 850 ml. per min. through check valves placed as close as possible to the fermenter. The controller sensitivity and the pressure switches were set so that the pumps were turned on when the deviation between the recorded pH and the set point was 0.05 and off when it was 0.02. The cycle controller was set to give a pumping time of 12.8 sec., which caused a change in pH of approximately 0.05. Since, with the flow system of measurement, there was a lag of 2.5 min. until the pH recorder reached equilibrium, the cycle time was set at three minutes. Although changes in pH were recorded almost immediately with the immersion electrodes, the same cycle and pumping times were used.

## OPERATIONAL METHODS

*Sterilization of Equipment*

All equipment, piping, and auxiliary vessels were steam sterilized at 10–15 p.s.i. for 15–18 hr. before use. Sterile air was obtained by passing compressed air through steam-sterilized glass wool filters (2).

*Preparation of Mash*

The continuous cooker unit was started with water only, which was discharged into a vessel not shown in Fig. 1. When the operating variables had

* *Beckman Instruments, Inc., South Pasadena, Calif. No. 8990-90 and No. 8970-92.*
† *Eastern Industries, Inc., New Haven, Conn. Model VW-1.*

been brought to the desired values, the flow of molasses to the slurry tank was started. About 1200 lb. of molasses* (51–57% sugar) was diluted to give 880 gal. of mash with a sugar concentration of approximately 7.25%. About one minute after the molasses flow was started, the steam pressure in the fermenter was reduced to atmospheric and the flash cooler effluent was switched to the fermenter. Initial pH (6.2 for *A. aerogenes* and 6.0 for *P. hydrophila* (1)) was adjusted by adding 80% acetic acid to the slurry tank with a small proportioning pump and, at half-hour intervals, phosphate was added by hand. For *A. aerogenes* fermentations, 4 lb. of triple superphosphate was added to give a phosphorus pentoxide concentration of 0.02%. For *P. hydrophila* fermentations, 1.7 lb. of dibasic ammonium phosphate was added to give a phosphorus pentoxide concentration of 0.01% (1). To ensure that all of the molasses, phosphate, and acetic acid were flushed into the fermenter, the rates of addition to the slurry tank were such that the required amounts of these ingredients had been added about an hour before the mash in the fermenter reached the desired volume. Thus, during the last hour, only water and steam were added. When the mash reached the desired level in the fermenter, the flash cooler effluent was switched from the fermenter to the vessel used in the starting up period and the unit was shut down.

Operating conditions and rates of flow for a typical run in the continuous sterilizer are given in Table I. The mash remained in the cooker 1.7 min. at a

TABLE I

TYPICAL OPERATING CONDITIONS FOR CONTINUOUS COOKER

| | Temperature, °C. | Pressure, p.s.i. | Flow rate, lb. per hr. |
|---|---|---|---|
| Water | 3.3 | — | 993 |
| Molasses | — | — | 320 |
| Steam | — | 50 | 175 |
| Raw mash | 80 | — | 1488 |
| Steam to cooker | — | 172 | 295 |
| Cooker inlet | 175 | — | 1783 |
| Cooker outlet | 163 | 120 | 1783 |
| Vapor | 86.4 | 12 in. Hg. vac. | 253 |
| Cooled mash | 86.4 | — | 1530 |
| Water to condenser | 27.5 | — | 42.2* |
| Water from condenser | 33.5 | — | — |
| Make-up water | 3.3 | — | 8.4* |

*Gal. per min.

velocity of 1.47 ft. per sec. The nominal retention time in the slurry tank was 11.5 min. About six hours was required for sterilization of one batch of mash not including one hour for starting up and 15 min. for shutting down.

The mash in the fermenter was cooled by circulating cold water through the coil and jacket as soon as the mash volume was sufficient to make cooling effective. Later, the temperature control system was turned on.

* *Canada and Dominion Sugar Company, Ltd., Chatham, Ont.*

Circulation of mash through the electrode flow chamber was started while the fermenter was being filled. When the mash had cooled to about 60° C., the flow chamber was by-passed and electrodes, sterilized by ultraviolet radiation (10, 18, 20), were inserted. The pH indicator and recorder were then put into operation. With the immersion electrodes it was necessary only to turn on the instruments. With either system of measurement, the pH indicator was initially set at the pH level determined on a sample with a laboratory instrument.

*Fermentation of the Mash*

The mash was allowed to stand overnight, then the pH was adjusted by manual operation of the acid and base pumps and 12 liters of inoculum was added. Foaming was controlled by adding approximately 800 ml. of sterilized corn oil (12) with the inoculum and, in some runs, an additional 800 ml. at 12 hr. For the first 24 hr., the air flow was set at approximately 3.6 c.f.m. per 1000 gal. Aeration was reduced for the next 12 hr. to about 1.8 c.f.m. per 1000 gal. and was cut off at 36 hr. The pH control system was generally operated to add only ammonium hydroxide, the pH being allowed to rise above the set point but not to fall below it. After 36 hr., when the fermentation was practically finished, the control system was turned off. Throughout the fermentation, samples were taken every 12 hr. for chemical analysis and restandardization of the pH instruments. At 48 hr., or when the fermentation was complete, the mash was transferred to a beer well.

## RESULTS AND DISCUSSION

*Sterilization*

Only one set of time and temperature conditions was used for the sterilization in the continuous cooker but these gave satisfactory results and were reasonable conditions to use in a pilot or large-scale plant. Before the continuous sterilizing system was installed, batches of mash were sterilized in the fermenter. Microscopic examination of these batches, just prior to inoculation, frequently showed traces of contamination but, with one exception, the diol yield was not affected. Mashes sterilized in the continuous cooker did not show initial contamination. From an operational standpoint, the two methods of sterilizing were about equal. The continuous cooker required more care and attention during the starting-up period but little attention during operation. Continuous sterilization, however, is ideally suited to a commercial plant where the cooled mash could be switched from one fermenter to the next without shutting down the system. Pfeifer and Vojnovich (11) report other advantages of continuous sterilization over the batch method.

*Fermentation*

Table II gives the results for 19 of the later fermentations. Except where noted, the data were obtained at 48 hr. For the runs that required a longer period for completion, the aeration time was extended. The sugar fermented

TABLE II

FERMENTATION RESULTS

| Run No. | Molasses | Sugar used, % | Fermentation efficiency, % | Lb. per 1000 lb. molasses | | | Ratio* | Air, c.f.m. per 1000 gal. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Diol | Acetoin | Ethanol | | 0–24 hr. | 24–36 hr. |
| *A. aerogenes* | | | | | | | | | |
| 1 | 1949 (6) | 98.5 | 83.7 | 167.5 | 9.8 | 31.9 | 5.55 | 3.5 | 2.0 |
| 2 | | 98.6 | 96.6 | 183.4 | 2.9 | 55.1 | 3.38 | 3.4 | 1.7 |
| 3 | | 99.3 | 86.5 | 156.2 | 3.5 | 56.6 | 2.82 | 3.4 | 1.7 |
| 4 | | 99.3 | 88.6 | 174.1 | 3.7 | 43.6 | 4.08 | 3.6 | 2.1 |
| 5 | | 97.9 | 87.1 | 180.3 | 11.5 | 25.9 | 7.44 | 3.4 | 1.7 |
| 6 | | 97.9 | 94.9 | 187.5 | 6.7 | 43.1 | 4.51 | 3.1 | 1.9 |
| 7 | 1949 (7) | 98.4 | 77.0 | 156.4 | 7.5 | 25.5 | 6.43 | 3.1 | 2.1 |
| 8 | | 95.9 | 94.4** | 160.3 | 13.3 | 52.8 | 3.29 | 2.8 | 1.6 |
| 9 | 1950 (8) | 98.9 | 84.1 | 144.2 | 1.0 | 65.1 | 2.23 | 3.2 | 1.9 |
| 10 | | 98.9 | 82.7 | 139.6 | 2.7 | 64.4 | 2.21 | 3.3 | 1.8 |
| 11 | | 99.9 | 82.6 | 142.8 | 0.9 | 62.8 | 2.29 | 3.5 | 1.9 |
| *P. hydrophila* | | | | | | | | | |
| 12 | 1949 (7) | 98.7 | 98.1 | 153.6 | 4.4 | 87.3 | 1.81 | 2.0 | 2.0 |
| 13 | | 98.5 | 86.1 | 152.2 | 5.1 | 58.0 | 2.71 | 3.3 | 2.3 |
| 14 | | 97.2 | 89.9 | 163.6 | 12.5 | 48.5 | 3.63 | 6.3 | 2.7 |
| 15 | 1950 (8) | 93.8 | 76.2*** | 148.4 | 0.3 | 41.7 | 3.57 | 3.6 | 1.8 |
| 16 | | 99.8 | 90.2 | 162.1 | 2.4 | 60.9 | 2.70 | 3.9 | 3.9 |
| 17 | | 99.1 | 87.3 | 147.6 | 1.3 | 69.2 | 2.15 | 3.3 | 3.3 |
| 18 | | 98.5 | 80.6 | 156.0 | 15.2 | 30.3 | 5.65 | 3.6 | 1.9 |
| 19 | | 96.8 | 96.7** | 161.0 | 25.3 | 55.3 | 3.37 | 3.6 | 1.6 |

* *(Diol + acetoin)/ethanol.* ** *60 hr.* *** *72 hr.*

varied from 93.8 to 99.8% and the over-all efficiency from 76.2 to 98.1%. The factors responsible for the variation in efficiency are unknown. The data do not show which organism or which molasses batch gave best diol yields, although No. 6 molasses appeared to give a consistently higher yield.

The ratio of diol plus acetoin to ethanol varied from 1.81 to 7.44. The yield of acetoin varied from 0.3 to 25.3 lb. per 1000 lb. of molasses or from 0.2 to 15.7% of the diol plus acetoin. It is known from laboratory experiments (7, 12, 20) that aeration decreases the production of ethanol (increases the ratio) and increases the production of acetoin. However, except for fermentation of No. 7 molasses with *P. hydrophila*, it was impossible to demonstrate this effect in pilot plant fermentations. In general, acetoin yield did not correlate with ratio and neither of these correlated with air rate. A possible explanation of the variation of product distribution is discussed in the section on foaming.

Table III gives data showing the average rate of fermentation of the six runs with No. 6 molasses and *A. aerogenes*. Similar results were obtained with the other batches of molasses.

TABLE III

RATE OF FERMENTATION
(Runs 1–6 of Table II, data expressed as gm. per 100 ml. of mash)

| Time, hr. | Diol plus acetoin | Acetoin | Diol | Ethanol | Sugar |
|---|---|---|---|---|---|
| 24 | 1.71 | 0.121 | 1.59 | 0.464 | —— |
| 36 | 2.74 | 0.131 | 2.61 | 0.668 | 0.13 |
| 48 | 2.81 | 0.098 | 2.71 | 0.663 | 0.11 |

*Foaming*

During the first 12 hr. of all fermentations, a very light foam, consisting of large bubbles, built up around the breaker and occasionally was carried out the exhaust gas outlet. (The liquid carried over as foam, which was broken in passing through the exhaust gas system, was collected and its volume added to the volume of fermented mash to obtain the final volume for calculation purposes.) This carry-over of foam would have been reduced by mounting the breaker directly below the exhaust gas outlet or by extending the exhaust gas pipe to a point inside the breaker.

In the runs to which a second 800-ml. batch of corn oil was added at 12 hr. (Nos. 9 to 17, Table II) the foam density and level remained about the same until 24 hr. In the remaining runs, to which only the initial batch of corn oil was added (Nos. 1 to 8, 18, and 19), a much denser foam was formed between 12 and 24 hr. and a large portion of the mash was in the form of foam. Additions of corn oil to this dense foam reduced the load on the agitator motor but did not appreciably lower the foam level, which the breaker established at, or slightly above, the bottom of the breaker.

The level of both types of foam decreased to below the breaker when the air flow was reduced at 24 hr. and disappeared when the air was shut off at 36 hr.

Because of smaller diameter bubbles, the denser foam gave more effective aeration than the lighter foam. The average ratio of the runs with the lighter foam was 2.59, whereas the average of the runs with the denser foam was 4.65. Also, the average acetoin yield of the first group was 3.4 lb. per 1000 lb. of molasses compared with 9.9 for the second group. The differences between these averages are statistically significant. The average air rates for the two groups were essentially equal.

Within the two groups, it is likely that the variation in product distribution resulted from differences in effective aeration. Effective aeration would be influenced by air rate, pH level, surface-active agents in the molasses, and the initial amount of corn oil. It is also possible that factors other than effective aeration were partially responsible for the variability of ratio and acetoin yield.

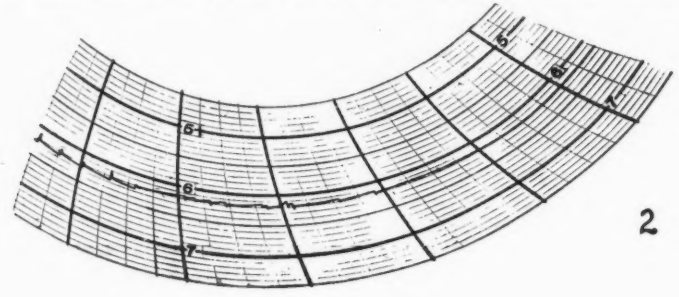
*Measurement and Control of pH*

The pH of all fermentations rose to about 6.6–6.8 in 6–8 hr. In 11 fermentations, the pH then decreased to the set point where it was maintained by

addition of ammonium hydroxide until 36 hr. when the pH control system was turned off. Occasionally the pH did not decrease to the set point. Six fermentations showed a second rise in pH between 24 and 36 hr. following a period of addition of the base. The amount of base added varied from 5.7 to 21.8 liters, the average amount being 14.1 liters.

Flow electrodes were used for the first 8 runs in Table II and immersion electrodes for the remaining 11 runs. With the flow electrodes, the average error (difference between pH on the recorder chart and the pH determined in the laboratory) was 0.10 for 23 fermentations. The average error for 13 fermentations with the immersion electrodes was 0.08.

The immersion pH electrodes were much easier to use than the flow electrodes. With the former, a separate ultraviolet sterilization was unnecessary and electrodes did not have to be put into sterile mash. Use of immersion electrodes eliminated the need for the circulating pump and its attendant piping, thereby reducing initial cost. Also, although no difficulties were encountered, a pump is a likely source of contamination. In addition to these advantages, there was a negligible time lag in measuring pH with the immersion electrodes as compared with a 2.5 min. lag with the flow electrodes.



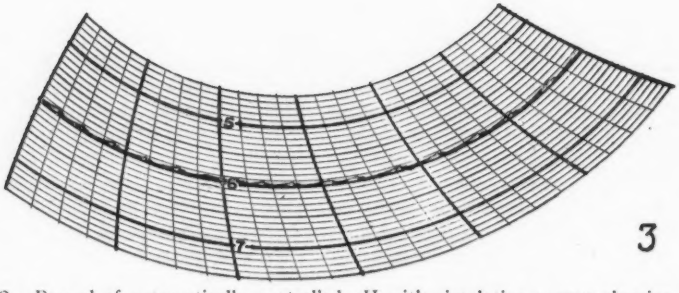




FIG. 2. Record of automatically controlled pH with circulating system showing change from addition of acid to addition of base. Time is read from left to right with one hour between heavy lines.

FIG. 3. Record of automatically controlled pH with immersion electrodes. Time is read from left to right with one hour between heavy lines.

Fig. 2 shows a pH record obtained with the flow electrodes. Both acid and base were controlled and the change-over from the addition of acid to an addition of base can be seen near the first column of figures. Fig. 3 is a similar photograph of a record of pH measured with the immersion electrodes. With these, there was no overpeaking following each addition of reagent. The overpeaking, however, did not affect the control response because equilibrium was attained before the end of the 3.0 min. cycle.

The pH control system, although it gave a "saw-tooth" line slightly to one side of the set point, was a sensitive system capable of handling very large load changes without risk of cycling or drifting from the set point. Because it was possible to set the acid and base switches independently, the neutral zone could have been set at any desired width. This would be advantageous where close control of pH is not necessary but where variation beyond certain limits should be avoided.

### *Temperature Control*

During periods of rapid fermentation, the mash temperature went as much as 5–6° C. above the water temperature. This could have been prevented by using the mash temperature to regulate the steam and cold water to the reservoir. This method of control, however, might cause cycling and overshooting. The ideal method would be to have the set point of the water temperature controller automatically set by the mash temperature. In the pilot plant this adjustment was done manually.

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# INCREASING THE FOOD VALUE OF WHEY BY YEAST FERMENTATION

## I. PRELIMINARY STUDIES ON THE SUITABILITY OF VARIOUS YEASTS[1]

By V. E. Graham[2], D. L. Gibson[3], H. W. Klemmer[4], and J. M. Naylor[4]

ABSTRACT

The possibilities of using various yeasts for increasing the food value of cheese whey have been investigated. The following cultures were studied: *Candida krusei*, *Torula utilis*, *Torula utilis thermophilus*, and *Torula cremoris*. *Torula cremoris* was the most suitable for commercial consideration. Highest cell counts were obtained when a 5–10% inoculum was made into an aerated medium. Supplementary nitrogen added in the form of ammonium salts was relatively effective in stimulating growth. The optimal pH and temperature requirements for the maximum production of cells in 72 hr. were 4.4–6.8 and 25°–30° C. respectively.

### INTRODUCTION

Whey utilization has presented a problem to the dairy industry for many years. Low in actual food value by volume, it has limited commercial use as a human or livestock feed. Where cheese factories are operating, the whey is often taken back by the producer for a low value livestock feed, but its disposal is more frequently accomplished by dumping. The latter procedure frequently involves an increase in sewage disposal cost. Methods of increasing the value of whey to a point where its disposal costs are met are therefore highly desirable. During 1950 and 1951 only about 10% of whey was converted to dry whey powder. Any additional process, therefore, which can convert whey to a profitable product would benefit the dairy industry.

Rogosa, Brown, and Whittier (10) carried out extensive investigations with lactose fermenting yeasts in search of a feasible means of utilizing whey for ethyl alcohol production. These authors reported that yields of alcohol averaging 90.73% of the lactose were obtained when *Torula cremoris* was used. A closely related yeast, *Torula utilis*, was used in Germany during the second World War to produce "Food Yeast" from both whey and waste sulphite liquor (3, 12). Still further references pertaining to the use of yeasts as "converters" of waste organic material are available in the literature. Rosenquist (11) outlined a Swedish yeast process and referred to the production of both alcohol and food yeast from waste sulphite liquor. Investigations by Kuorth (5) and Peterson *et al.* (8) on the production of fodder yeasts from wood sugar showed that the yield of *Torula utilis* in some cases exceeded 50% of the weight of sugar consumed. Consequently, an investigation was under-

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taken to determine whether the commercial value of Canadian cheese whey could be increased by fermentation with yeast. This report deals with the preliminary tests carried out to ascertain the most suitable yeasts for the process and the most favorable conditions for selected yeast growth.

## METHODS AND MATERIALS

### (*a*) *Cultures of Microorganisms Used*

*Candida krusei*
*Torula cremoris* (University of Saskatchewan stock culture collection)
*Torula utilis* (American type culture collection)
*Torula utilis thermophilus* (American type culture collection)
*Streptococcus lactis* (University of Saskatchewan stock culture collection)
*Lactobacillus bulgaricus* (University of Saskatchewan stock culture collection)

The yeast cultures were maintained on Sabouraud's agar slants (2) and were transferred regularly to maintain viability. Several days before the initiation of a trial run, the culture was transferred to a flask of sterile whey and was incubated at a temperature suitable for the culture concerned. The bacteria were cultured in tubes of sterile litmus milk.

Growth characteristics and preferences of each yeast culture were studied while grown individually, associatively, and in associative sequence with another yeast or with lactose fermenting bacteria.

### (*b*) *Preparation of Whey*

Unless otherwise indicated, the whey used during these experiments was prepared by rennet coagulation of fresh skim milk. Both laboratory and factory produced whey was investigated, the former, however, was preferred because of the ease with which batches could be prepared. During certain trials "acid whey" was used as the growth medium, and was produced by methods normally followed in cottage cheese manufacture. The whey was either sterilized at 250° F. for 20 min., or was pasteurized at 143° F. for 30 min.

When supplementary nitrogen was used it was added to the medium in the form of various inorganic salts. The identity of the nitrogen source in each case is indicated in the results. The pH of the medium was adjusted to the desired level by the addition of hydrochloric acid, sodium hydroxide, sulphuric acid, and sodium bicarbonate solutions.

Aeration was accomplished in various ways. During the earlier trials, air was either blown in from an air pressure line, or was drawn in through a vacuum by an aspirator attachment. In subsequent trials a small-scale laboratory fermenter described by Klemmer (4) was employed in which the air was drawn in and dispersed by rotating impellers.

### (*c*) *Chemical and Bacteriological Analyses*

Cell production was used to measure the effect of different environmental conditions. Estimations of cell concentration were obtained by three different methods.

1. Standard plating procedure on Sabouraud's agar (1).
2. Direct microscopic count of appropriate dilutions using the Newman-Lampert stain (1).
3. Counting chamber of a haemacytometer type (1).

The latter method gave the most consistent results and therefore was adopted as standard procedure during these investigations.

The lactose content of the whey was determined by the modified Fehling technique described by Richmond (9). Micro-Kjeldahl determinations were made to estimate the nitrogen content of the substrate both before and after fermentation (9). Fat and total solids determinations were made on a Mojonnier tester (6).

## RESULTS

Two aspects of whey fermentation were considered during these trials, (1) an attempt was made to determine the suitability of any given yeast culture for commercial application; and (2) an investigation was made of the environmental preferences of these cultures. Since space does not permit a complete discussion of each aspect of this work, only a brief summary of many trials conducted over a considerable period of time will be presented here. Four different cultures were studied, certain tests being repeated in each case. Unless otherwise stated, any trend established for one culture can also be considered applicable to the other cultures.

### *Candida krusei*

This organism was unable to utilize lactose as an energy source and therefore was grown in association with a culture of *Streptococcus lactis*. Both organisms were inoculated into sterile whey, and trials were carried out to determine the effect of aeration and size of inoculum on cell production. The results of these trials are presented in Table I. Similar phenomena were observed during the studies of the other yeast species. Aeration stimulated growth only after the cultures were at least 24 hr. old. Similarly, inocula ranging from 5–10% were found to be most effective in producing high cell counts within a relatively short time.

TABLE I

THE EFFECT OF AERATION AND SIZE OF INOCULUM ON THE GROWTH OF *Candida krusei* IN ASSOCIATION WITH *Streptococcus lactis* IN STERILE RENNET WHEY AT 25° C.

| Time after inoculation, hr. | Microscopic counts per ml. × $10^6$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1% | | 3% | | 5% | | 7% | |
| | Aer.* | Nonaer.** | Aer. | Nonaer. | Aer. | Nonaer. | Aer. | Nonaer. |
| 24 | 2.8 | 4.0 | 3.9 | 4.2 | 5.0 | 7.0 | 6.1 | 8.6 |
| 48 | 21.4 | 10.1 | 28.4 | 11.7 | 31.9 | 16.0 | 40.4 | 17.2 |
| 72 | 36.9 | 13.8 | 44.3 | 16.2 | 40.4 | 18.4 | 47.4 | 20.2 |
| 96 | | | | | 77.1 | 18.5 | 82.5 | 21.2 |
| 120 | | | | | | | 103.2 | 27.9 |

*Aer.—aerated. **Nonaer.—nonaerated.

The maximum yield of *C. krusei* obtained from normal whey was 18.0 gm. per gallon. The dried yeast whey averaged 58.1% protein.

*Torula utilis thermophilus*

This organism also was unable to utilize whey lactose as an energy source and required the associative growth of an active lactose fermenter. Since the optimum temperature for this yeast, 38° C., was too high for *S. lactis*, *Lactobacillus bulgaricus*, a thermophilic lactic acid bacterium, was added to the whey medium. An investigation of the temperature and pH preferences of this organism, and further of its inability to proliferate effectively in whey, indicated that *T. utilis thermophilus* was unsuitable for the purposes of these experiments.

*Torula utilis*

The success reported in the use of this organism to produce food yeast from molasses, waste sulphite liquor, and wood sugar prompted an investigation of the ability of *Torula utilis* to grow in whey. Skoog (12) indicated the advisability of adding supplementary nitrogen to the medium, and the optimum quantities required by this organism appeared to be in the order of 2.5 to 3.0 gm. inorganic salt per liter of whey. The data obtained from a trial run using this culture are presented in Table II.

TABLE II

GROWTH OF *Torula utilis* IN WHEY CONTAINING 2.83 GM. AMMONIUM SULPHATE AND 2.57 GM. UREA PER LITER. TEMPERATURE, 26° C.

| | 0 hr. | 8 hr. | 24 hr. | 32 hr. | 48 hr. | 96 hr. |
|---|---|---|---|---|---|---|
| Microscopic count per ml. $\times 10^6$ | 25.5 | 260.0 | 318.0 | 314.0 | 662.0 | 1316.0 |
| pH | 4.58 | 7.68 | 7.7 | 4.0* | 4.0 | 4.3 |
| % lactose | 3.53 | 3.53 | 3.53 | 3.39 | 0.25 | — |

* *pH reduced by addition of acid.*

Yeast growth during the first 24 hr. occurred primarily at the expense of the lactic acid present, as evidenced by the increased pH and the constancy of the lactose content. The rapid depletion of lactose after the first 24 hr. was shown by microscopic observation to be due to bacterial contamination. The rapid conversion of lactose to lactates also accelerated yeast multiplication considerably, but these results are not valid owing to the contamination which occurred. It became evident after further experimental runs with this organism that *Torula utilis* was unsuitable for commercial whey fermentation owing to its inability to metabolize lactose as such.

*Torula cremoris*

Of the four organisms studied, *Torula cremoris* was the only one which could utilize whey lactose as a source of energy. Consequently, a study was

made of several factors which might influence the growth of this particular culture in whey. It appeared that the sterilization exposures used during autoclaving might have destroyed or modified certain growth factors naturally present. To verify these presumptions, duplicate trials were conducted using both pasteurized and sterilized media. Similarly, the effect of acid whey was compared to normal rennet whey. Finally trials were performed to determine a suitable incubation temperature. The results from these trials are summarized in Table III.

TABLE III

THE EFFECT OF AUTOCLAVING, TEMPERATURE, AND ACID WHEY ON THE GROWTH OF *Torula cremoris*

| Type of whey | Temperature, °C. | Microscopic count × $10^6$ | | | |
|---|---|---|---|---|---|
| | | 24 hr. | 48 hr. | 72 hr. | 96 hr. |
| Sterilized rennet | 26 | 24.5 | 68.7 | 87.5 | 116.0 |
| Sterilized rennet | 37 | 24.8 | 64.3 | 69.4 | 84.2 |
| Sterilized acid | 26 | 22.5 | 33.5 | 62.5 | 46.0 |
| Sterilized acid | 37 | 9.0 | 5.0 | 8.2 | 4.0 |
| Pasteurized rennet | 26 | 17.2 | 76.5 | —— | 134.5 |
| Pasteurized acid | 26 | 13.2 | 61.5 | —— | 80.0 |

It is evident that pasteurized rennet whey was the most suitable medium and that 26° C. was the most suitable temperature for propagation. Since all of these conditions can be readily satisfied during commercial operations, more detailed investigations were undertaken on this organism. It was found that pH 4.4 was the optimum for growth, and that slight stimulation was effected by the addition of 2.5 gm. of dibasic ammonium phosphate per liter. Yeast growth also was stimulated by efficient aeration of the whey, whereas alcohol production was enhanced by partial anaerobiosis. A series of fermentation tests using various sugars indicated *T. cremoris* preferred lactose over glucose, galactose, fructose, *d*-mannose, sucrose, maltose, or raffinose in the growth medium.

## DISCUSSION

The cultural characteristics of the four cultures studied showed that *Torula cremoris* was more suitable for whey fermentation processes than either *Candida krusei*, *Torula utilis thermophilus*, or *Torula utilis*. The chief disadvantages of the latter cultures lie in their inability to utilize lactose and thus require the associative growth of a lactose fermenter. *Torula cremoris* proliferated excellently in whey and its "sugar pattern" showed it to prefer lactose to other sugars as energy sources. Further, the ability of this organism to grow well at 26° C. would be advantageous from the commercial point of view. The trials carried out on the other cultures, however, produced valuable data and the results obtained during those trials were often applicable to *Torula cremoris*. It is particularly noteworthy to consider the various aspects of associative growth evidenced during some of the experiments. A culture of

*S. lactis* or *Lactobacillus bulgaricus*, for example, has a relatively low viability in whey medium. The presence, however, of a yeast considerably prolonged the activity of these organisms. It is possible that the B vitamins synthesized by the yeasts were responsible, or perhaps other growth factors were elaborated to support the growth of the lactic acid bacteria.

Two points of interest arose during the trials with different types of whey. It was found that whey produced during rennet coagulation was preferred to whey produced by acid precipitation, and that the yeast cultures preferred pasteurized, rather than sterilized, whey as a growth medium.

## SUMMARY AND CONCLUSIONS

1. In an attempt to increase the food value of cheese whey by yeast fermentation, certain preliminary investigations were carried out to find a suitable yeast culture and to determine its environmental preferences.

2. Four yeast cultures were studied: *Torula cremoris*, *Candida krusei*, *Torula utilis*, and *Torula utilis thermophilus*. Of the four, *Torula cremoris* showed the most promise when grown under conditions simulating commercial conditions.

3. A detailed investigation of the environmental conditions necessary for the successful propagation of this organism in whey showed the following:

(*a*) The best medium for growth was pasteurized whey produced by rennet coagulation of skim milk. Aeration, though not effective during the initial stages of growth, considerably enhanced the production of cells after 24 hr.

(*b*) The size of inoculum which gave the most promising results was 5–10% of a 24-hr.-old whey culture of the yeast.

(*c*) 25–30° C. was the best temperature for the production of the highest number of cells in 96 hr.

(*d*) Supplementary nitrogen added in the form of inorganic ammonium salts or urea stimulated growth.

(*e*) The optimum pH for the development of this organism in whey was 4.4. However, good cell yields were also obtained when the pH ranged from 5.9 to 6.8.

4. Fermentation of cheese whey with an organism like *Torula cremoris* might be useful in converting low value whey into a high biological value animal food supplement.

## ACKNOWLEDGMENT

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# INCREASING THE FOOD VALUE OF WHEY BY YEAST FERMENTATION

## II. INVESTIGATIONS WITH SMALL-SCALE LABORATORY FERMENTERS[1]

By V. E. Graham[2], D. L. Gibson[3], and H. W. Klemmer[4]

ABSTRACT

A description is given of two small-scale laboratory fermenters which can be used for the rapid fermentation of cheese whey by yeasts. *Torula cremoris*, when grown under optimum conditions, removed the lactose from the whey and simultaneously increased its protein and riboflavin content. The commercial possibilities of this process are noted.

## INTRODUCTION

Natural whey may be processed in various ways to increase its value. Among the methods used today are: spray and roller drying to produce whey powder, fractionation for the preparation of albumin and globulin proteins, and concentration and powdering of the lactose. Fermentation reactions might provide an additional means of commercial whey utilization. The objects of fermentation are threefold: (*a*) to remove lactose in order to facilitate drum drying, (*b*) to increase the nutritional value of whey by yeast synthesis of proteins and vitamins, and (*c*) to develop a method of whey disposal which would be profitable to the cheese industry.

In a previous paper, Graham *et al.* (3) outlined the results of a study made on the suitability of various yeasts for whey fermentation. It was reported that *Torula cremoris* grew well in pasteurized rennet whey and these authors suggested the possibility of using this culture on a commercial basis. However, before pilot plant operations could begin, it was necessary to undertake some preliminary investigations with a laboratory-scale fermenter.

Thaysen (7) described the operation of a 10-liter laboratory fermenter. He pointed out the necessity of adding nutrients at a rate determined by the logarithmic growth rate of the yeast. A small-scale fermenter was also described by Peterson *et al.* (6). This apparatus consisted of a 5-liter Pyrex flask set in a constant temperature water bath. The medium was aerated by forcing air through a canvas bag submerged below the liquid surface. Kuorth (5) used fritted glass dispersion tubes for the same purpose. Feustel and Humfeld (2) built a fermenter from a tall glass tube, 3 in. in diameter. Propellers mounted on a central shaft agitated the liquid and dispersed the air supplied from a pressure line. In a later experiment, Humfeld (4) modified this apparatus so that one set of propeller blades created a vortex type of current in the medium and thus drew air in by suction.

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It was the object of these investigations to construct an apparatus, similar to the ones mentioned above, for the purpose of propagating yeasts in whey. This report describes the results obtained with two of these laboratory fermenters.

## MATERIALS AND METHODS

### 1. *Cultures of Yeasts Used*

Two strains of *Torula cremoris*, one isolated at the University of Saskatchewan and the other at the Iowa State College, Ames, Iowa, were used during these trials. The cultures were propagated in sterilized whey and were transferred every 48 hr. to maintain viability.

### 2. *Preparation of Whey*

Both factory- and laboratory-prepared wheys were used. Whey received from cheese factories usually had an initial pH of 4.5 and varied widely in its fat content. Laboratory whey was prepared by the rennet coagulation of fresh skim milk and had an initial pH of 6.0. The lactose content of the factory whey was usually 1% lower than that of the laboratory whey. The whey used for maintaining the stock cultures was sterilized at 250° F. for 20 min., whereas the whey destined for trial runs in the laboratory fermenter was pasteurized at 143° F. for 30 min., cooled, and stored under refrigeration.

### 3. *Preparation of Inocula*

Twenty-four hours prior to the initiation of a trial run, 100 ml. of a stock culture were added to fresh pasteurized whey to produce an active inoculum. The whey was placed in a small fermenter and aerated for 24 hr. At the end of this period the fermenter was filled to the desired level with fresh pasteurized whey and the experiment was started.

### 4. *Description of Laboratory Fermenters*

Model 1. This apparatus consisted essentially of a nickel plated bronze cylinder fitted with gasketed end plates. Aeration and mixing were accomplished by three impellers mounted on a central revolving shaft. The topmost impeller served as a foam breaker, the lower two provided whipping action and agitation. The maximum capacity of whey was about 2 liters.

Model 2. This was constructed from a glass cylinder 122 cm. long and 9.2 cm. in diameter (Fig. 1). The capacity was about 9 liters. The end plates, secured by metal tie rods, had spouts for draining, and for entrance and exit of air and an opening for a central agitator shaft. The brass agitator shaft was rounded at the bottom where it turned in an oilless bronze bearing. Impellers were mounted at regular intervals along this shaft to break the foam, to stir the mixture, and to emulsify the incoming air. The agitator was turned by a $\frac{1}{3}$ h.p. motor at approximately 100 r.p.m.

### 5. *Aeration*

Air was introduced into the fermenting medium either by positive pressure (12 lb. p.s.i.) or after evacuation with a vacuum pump. The air was broken

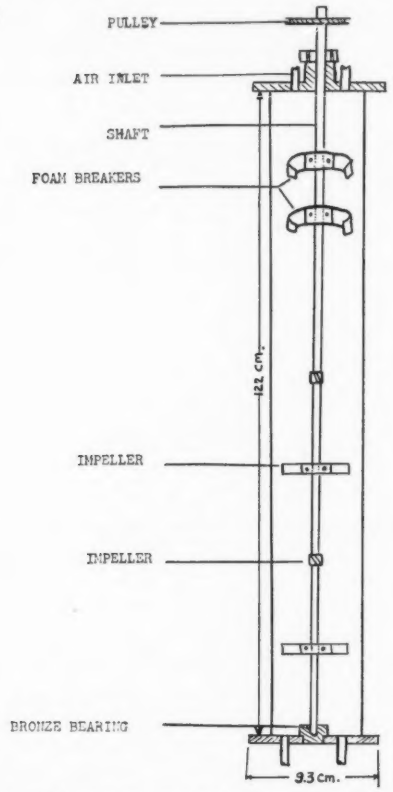


FIG. 1. Diagram of small-scale laboratory fermenter.

into fine bubbles by a submerged Berkefeld filter or by forcing it through a perforated copper tube.

6. *Bacteriological and Chemical Analyses*

A haemocytometer blood cell counting chamber was used in obtaining cell counts. One milliliter of the sample yeast suspension was diluted with 8.7 ml. distilled water and 0.3 ml. of Newman-Lampert stain. This dilution was satisfactory for all ranges of yeast concentration encountered during the study.

Chemical analyses of the whey and whey slurry were performed according to the methods previously outlined by Graham *et al.* (3). Riboflavin and thiamine contents were estimated by fluormetric methods (1).

## PRESENTATION OF DATA

A number of trials were undertaken with these fermenters in order to determine their suitability for growing yeast in whey. The results of several typical runs are summarized in Table I and Table II.

TABLE I

FERMENTATION OF PASTEURIZED CHEDDAR CHEESE WHEY BY *Torula cremoris* GROWING IN A LABORATORY-SCALE FERMENTER (MODEL 2).

| | 0 hr. | 12 hr. | 24 hr. |
|---|---|---|---|
| Cell population (per ml. × $10^6$) | 80.0 | 1270.0 | 1200.0 |
| Total solids | 6.1 % | 3.5 % | 2.7 % |
| Lactose | 4.2 % | 1.5 % | 0.1 % |
| Total solids less lactose | 1.9 % | 2.0 % | 2.6 % |
| Protein | 0.89% | 1.06% | 1.06% |

TABLE II

FERMENTATION OF PASTEURIZED CHEDDAR CHEESE WHEY BY *Torula cremoris* GROWING IN A LABORATORY-SCALE FERMENTER (MODEL 1)

| | 0 hr. | 24 hr. | 48 hr. |
|---|---|---|---|
| Cell population (per ml. × $10^6$) | 10.5 | 66.1 | 144.9 |
| Lactose | 5.00% | 1.85% | 0.15% |
| Protein | 0.71% | 0.72% | 0.78% |

The results show that growth occurred more rapidly in the large fermenter (Model 2) than in the small one (Model 1). This phenomenon was probably due to the more efficient aeration and dispersal system used in the former apparatus. A higher protein concentration was observed in whey processed in the larger fermenter but the change was relatively slight in the small apparatus. The highest yield obtained during any run was two billion cells per milliliter, equivalent to 2.5% yeast by volume and representing 25–30 gm. of yeast per liter of whey.

It was observed that the yeasts synthesized riboflavin during their growth. In Table III are presented the vitamin assays of several lots of whey before and after fermentation.

TABLE III

RIBOFLAVIN SYNTHESIS BY *Torula cremoris* GROWN IN PASTEURIZED WHEY

| Trial | Riboflavin content (mgm./100 ml.) | |
|---|---|---|
| | Fresh whey | After 48 hr. fermentation |
| 1 | .2215 | .2646 |
| 2 | .1538 | .2215 |
| 3 | .2000 | .2433 |
| 4 | .1233 | .2433 |
| Average | .1746 | .2431 |

A series of trials were conducted to test the efficiency of lactose utilization and cell production in dilute, concentrated, and normal whey. These results are summarized in Table IV.

Whey diluted to a lower lactose concentration allowed slightly more efficient utilization during the first 24 hr. than did normal whey. Whey concentrated to about double normal lactose content provided high final yeast yields but less efficient lactose conversion.

TABLE IV

THE EFFECT OF LACTOSE CONCENTRATION ON THE DEVELOPMENT OF *Torula cremoris* IN PASTEURIZED WHEY

| Type of whey | 0 hr. | 24 hr. | 48 hr. |
|---|---|---|---|
| Normal | | | |
| Cell population ($\times 10^6$ per ml.) | 17.3 | 645.9 | 1589.3 |
| Lactose (%) | 4.26 | 2.41 | 0.11 |
| Dilute | | | |
| Cell population ($\times 10^6$ per ml.) | 22.5 | 305.4 | 80.5 |
| Lactose (%) | 1.03 | 0.42 | 0.25 |
| Concentrated | | | |
| Cell population ($\times 10^6$ per ml.) | 8.5 | 42.4 | 1007.9 |
| Lactose (%) | 9.05 | 8.04 | 2.07 |

Cell production was found to increase directly with increased aeration whereas alcohol production varied inversely. The conversion of lactose under conditions of inefficient aeration was approximately 6.0% to yeast and 48.2% to alcohol by weight; under efficient aeration this ratio was reversed becoming 48.3% conversion to yeast cells and 12.8% conversion to alcohol.

## DISCUSSION OF RESULTS

The main object of these investigations was to develop a small-scale laboratory fermenter for the purpose of fermenting whey by yeasts. It can be seen from the results that the two apparatuses described in this paper were quite effective for this purpose.

The 2-liter fermenter was not as satisfactory as the 8-liter fermenter owing mainly to its size. Only very small quantities of whey could be tested in the former and the experimental error was accordingly magnified. Furthermore, the small quantity of fluid prevented efficient heat dissipation and some trouble was experienced in maintaining a constant temperature.

The 8-liter fermenter provided an excellent laboratory apparatus for the study of yeast whey fermentations. It was large enough to allow for variations in the total quantity of medium to be processed and yet small enough to permit easy manipulation. The glass walls allowed the operator to detect any technical difficulties as well as permitting semicontinuous operations to be carried out without complete dismantling.

The various tests performed with this fermenter greatly facilitated the planning of trial runs on a pilot plant basis and preliminary experiments could be performed with relatively small expenditures of medium, time, and labor.

## SUMMARY AND CONCLUSIONS

Two small-scale fermenters were constructed; one apparatus, consisting essentially of a glass container and a motor driven agitator, was particularly successful. Yeast counts up to one and one-half billion cells per milliliter were obtained with this fermenter. These yeasts utilized the lactose in whey efficiently and simultaneously increased the protein and riboflavin content.

Laboratory-scale fermentation of whey using *T. cremoris* was accomplished successfully. The results obtained might provide a commercial method for utilizing whey and reducing the problem of disposal.

## ACKNOWLEDGMENT

The writers are indebted to the National Research Council for making the funds available for this study.

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# AN IMPROVED RELAXOMETER FOR VISCOELASTIC SUBSTANCES APPLIED TO THE STUDY OF WHEAT DOUGH[1]

By J. R. Cunningham, I. Hlynka, and J. A. Anderson

ABSTRACT

An improved model of a previously reported relaxometer for continuous recording of the relaxation of stresses in a stretched dough is described. The speed of response of the recording pen has been increased so that its maximum excursion of 8 in. may be traversed in as little as 0.2 sec. The small lag of the pen has been observed by means of an oscilloscope and found to be negligible. Motion of the strain gauge in recording the tension is kept below 0.2 mm., which is within 1% of the strain, by a simple lever arrangement. The linkage connecting the strain gauge to the pen is a linear servomechanism and is free from the effect of fluctuations in line voltage. Relaxation curves have adequate reproducibility; they are replotted on a logarithmic time base and extend over six decades from $10^{-2}$ to $10^{4}$ sec. Curves are referred to a generalized Maxwell model having a distribution of relaxation times. The distribution function of relaxation times is obtained by differentiation of the relaxation curve. Increasing the water content of the doughs decreases the magnitude of the distribution function but does not markedly alter its shape, while increasing the rest period causes a greater concentration of relaxation times at the short time end. Decreasing dough protein content also appears to change the shape of the distribution function in a manner somewhat analogous to that of increasing water content.

Several instruments have been used in cereal laboratories for testing dough. Very few of them have been designed with a prior consideration of definable physical properties of the material to be tested (2). A few investigators (6, 7, 8, 9, 15), however, have made studies of certain physical properties such as viscosity and elastic modulus. Examination of this work shows that dough is a complex substance and that modulus and viscosity are in fact not definable in terms of simple concepts. Neither of these quantities may be specified uniquely, but require a complete curve as a function of time.

One such curve that is frequently used to relate viscoelastic properties to time is the relaxation curve, which describes the relaxation of stress at a constant extension. This procedure has been chosen as a means of studying wheat dough.

An instrument designed for observing stress relaxation has been briefly described in a previous paper (6). It has now been modified to meet requirements of increased speed and sensitivity of response. The range is sufficient for observation of relaxation times of 0.01 sec. up to several hours. The instrument is described in some detail as it may be of use in studies of other viscoelastic substances.

A number of experiments designed to determine the applicability of the machine, and the direction that subsequent research should follow, are described. Curves showing the relaxation of tension set up by extending samples of spring-wheat dough have been obtained for a range of six cycles of logarithmic time. An approximate distribution of relaxation times is obtained by

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differentiating these curves. The curves and the distribution functions derived from them have been examined from the standpoint of reproducibility and range of variations to be expected. Doughs from wheats of three different protein contents were used and widely varying rest periods and water contents have been examined.

## GENERAL DESCRIPTION OF RELAXOMETER

A photograph of the relaxometer is shown in Fig. 1. The principles of the machine are similar to those described in a previous paper (6). A ball of dough is impaled on a split pin dough holder. One half of the pin can be made to travel at a constant speed, thus extending the ball of dough. The other half forms part of a differential transformer strain gauge and is electronically linked to a kymograph so that the tensions built up in the dough may be recorded.

The rate of travel of the extension mechanism may be controlled, as may also the magnitude of the extension. The extension of the dough may thus be stopped either when a certain extension is reached or when preset tension has been built up.

The small movement of the strain gauge half of the dough holder is amplified by means of a pair of balanced differential transformers and a servomotor.

The recorder or kymograph is in the form of cartesion graph paper wrapped on a cylinder driven by a synchronous motor. This permits continuous recording of both the extension and relaxation parts of the curve. The drum may be driven at speeds of from one revolution in three minutes to one in two seconds.

The requirements of an instrument for studying the relaxation exhibited by dough are speed of response, sensitivity, and the ability to hold the sample at constant extension during relaxation. The speed of the recorder has been made high by keeping inertia effects at a minimum and by mounting all moving parts on ball bearings. The pen will make its full excursion of 8 in. in 0.2 sec. and will quite easily follow oscillations of 20 to 30 vibrations per sec. The maximum motion of the strain gauge is 0.25 mm. and is not more than 1.6% of the extensions involved.

## DETAILED DESCRIPTION OF PARTS

The relaxometer is built in four units: the sensing unit with the dough holders, extension mechanism, and strain gauge; the kymograph; the amplifier; and the power supply. These units are on chassis and may be separated from one another for repair or for use with other instruments. The description of the parts is given with the aid of the labelled photograph shown in Fig. 1.

### *Extension Mechanism*

One half of the split pin dough holder is mounted on a ball-bearing carriage which may be pulled by a cable and a drum operated by a synchronous motor. A microswitch which turns off the motor acts as a stop for the carriage. Adjust-

ment of the position of the switch permits extension of from 0 up to 5 cm. This range has been found adequate for the dough samples used, but greater extensions could be readily obtained by modifying the extension ways. The cable is attached to the carriage by means of a fairly strong spring to allow any coast of the motor to be taken up and so ensure instant stopping of the carriage. To stop extension at a preset tension, there is a relay arrangement tripped by the recorder pen which stops the extension motor and applies a set of brakes to the extension carriage. These brakes are loaded with a fairly powerful spring and are released by the discharge of an 80 $\mu$f. condenser through an electromagnet; this arrangement prevents interference with the amplifier. The extension motor is provided with a reversing switch to adjust the carriage position and a light spring returns the carriage as the cable is unwound. The rate of extension can be altered by providing winch drums of different diameters.

*Strain Gauge*

The strain gauge is provided by one section of the dough pin on a ball-bearing carriage connected to a spring by a lever arm. The modulus of the spring governs the stiffness of the gauge. The total pin motion is under 0.25 mm.

*Differential Transformers*

The differential transformer arrangement used is very similar to that used by Stein and Schaevitz (12) who give a very good description of it. In each transformer there are two secondary windings connected so that for a certain position of the movable core the output is zero. When the core is moved in the first transformer a signal from the secondaries results, the amplitude of which is proportional to the distance moved and the phase depends on the direction moved. The core of this first transformer (see Fig. 1) is connected by a lever to the strain gauge half of the dough pin. With this arrangement the movement of the core in the transformer is 10 times that of the movement of the dough pin.

A second identical transformer is electrically balanced against the first. The core of this is moved by a servomotor through a worm drive and a cam so as to cancel the signal caused by movement of the first core. The pen is connected to the camshaft by a cord and pulley. The amplification from strain gauge to pin is over 800.

*Kymograph*

The kymograph consists of the second differential transformer, the servomotor, the pen, and the graph. The graph paper is wrapped around a cylinder which is driven by a synchronous motor. The speed may be changed by adjusting the gearing between the motor and cylinder. The pen carriage is made of aluminum, is very light, and travels on three small ball-bearing assemblies. A master switch starts the extension motor, the kymograph, and the servomechanism. A graph is thus obtained for both the build-up and the decay of tension in the sample.

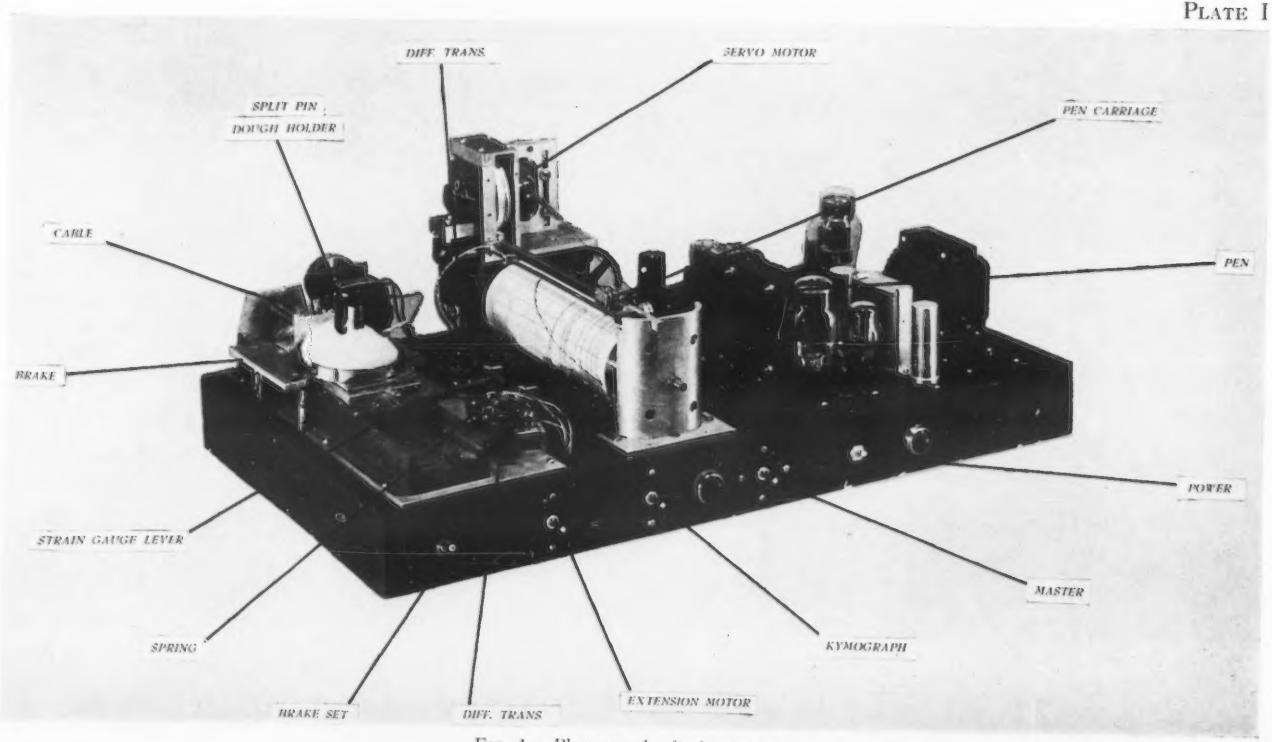


FIG. 1. Photograph of relaxometer.

*Power Supply and Amplifier*

The power supply is a standard type of regulated unit supplying up to 300 v. positive and 100 v. negative. A high degree of regulation is not required for this relaxometer but, as has been mentioned, the unit is removable from the relaxometer and may be used on other apparatus.

Two 60-cycle signals 180° apart are imposed on the plates of a 6AS7 tube. One of these signals is fed to the servomotor, depending upon the phase of the signal from the amplifier. The selection of the signal determines the direction of rotation of the servomotor.

## CALIBRATION AND PERFORMANCE

A graph drawn by the instrument is the load or tension built up in the dough versus time. The time is given by the speed of rotation of the kymograph cylinder, while the load is given by the amplification of the strain gauge-to-pen linkage and the stiffness of the strain gauge spring. The displacement of the pen from zero on the graph is calibrated in terms of grams of tension on the strain gauge pin and is shown in Fig. 2. The pen displacement from zero on

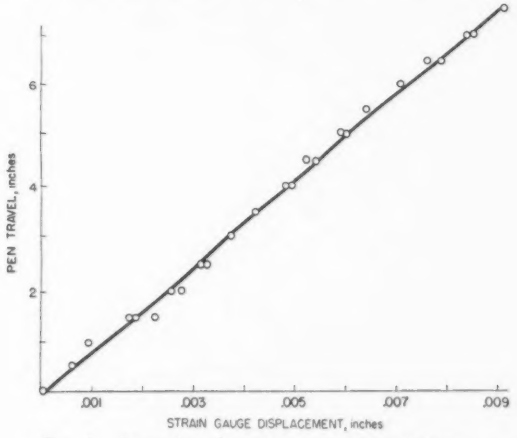


FIG. 2. Linearity of strain gauge to pen linkage.

the kymograph chart is plotted against the position of the strain gauge pin as measured by a micrometer. This plot shows the response of the mechanism to be linear.

The instrument was calibrated by hanging weights from the strain gauge pin by means of a cord and pulley. The pen displacement from zero was noted for a given weight. A calibration curve is not shown since it is the same as Fig. 2, if the strain gauge position scale be replaced by a scale of weights. The calibration may be changed within a wide range by selecting a proper spring for the end of the strain gauge lever.

Because of the nature of the strain gauge-to-pen linkage (and control mechanisms in general) the pen lags slightly behind the motion of the strain

gauge and has a tendency to overshoot and hunt. These effects appear on the curves obtained as an oscillation superposed on the true curve. The oscillation may be kept to a minimum by proper design but must be measured in order to determine its significance.

The response of the pen has been examined by mechanically imposing a "square" impulse on to the strain gauge. With the servomotor hunt control adjusted to give a minimum hunt oscillation yet retain a maximum pen speed, the oscillation has a period of about 0.3 sec. The amplitude however decreases by a factor of about 10 for each period. It is quite simple to "smooth out" this oscillation on the relaxogram that is drawn and to arrive at the relaxation curve.

The "resonance" vibration of the strain gauge and strain gauge lever has a period considerably smaller than the relaxation times measured and so is not bothersome.

A second method of examining the response of the pen has been used. The signal output of the two differential transformers is proportional to the relative positions of the two transformer cores. This signal is then, in effect, the lag of the pen behind the strain gauge pin. This signal may be readily observed by means of an oscilloscope while the relaxograms are being drawn and thus the lag may be measured *in situ*. The lag is greatest at the beginning of extension and of relaxation, but is rapidly damped down to a negligibly small value. The lag has been observed for a number of curves, and the effect on the relaxation function is that during the first plotted cycle of logarithmic time the curve should be at most 3 or 4% lower. This corresponds to a time uncertainty of approximately 0.06 sec.

## EXPERIMENTAL TESTS OF THE RELAXOMETER

A number of experiments have been conducted on dough to test the behavior of the instrument. The type of curve obtained is described below along with the reproducibility of that curve under a given set of conditions. Curves are also presented for doughs in which water content, rest period, and protein content, were varied.

### *Relaxation Curve*

Water is added to flour in the desired proportions to give 150 gm. of dough. The dough is mixed, and rounded, molded onto the dough holder and allowed a "rest period" before being stretched on the relaxometer.

The type of curve obtained is basic to the study and is shown in Fig. 3. The ordinate is the tension on the dough in grams and the abscissa is the time base given by the continuous revolution of the kymograph drum. The upper curve, which shows both the extension curve rising to maximum tension and the relaxation or decay curve, represents one complete revolution of the kymograph drum; the lower curve is obtained in the second revolution; while the short interrupted parts of the curve are obtained at desired time intervals for the remainder of the relaxation process.

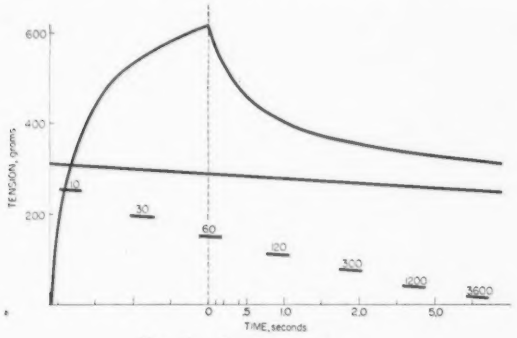


FIG. 3. A relaxogram.

The relaxation part of the curve is replotted on semilog paper, tension vs. log-time, in Fig. 4. The time when extension ceases is taken as zero time for this plot, and the so-called relaxation function is sigmoidal in shape and is characterized by a long and fairly linear region of inflection. The curve illustrated extends over six cycles of logarithmic time and is fairly linear for more than three of them. This linear behavior was noted previously (6) and is similar to that observed in other polymeric materials (3, 4).

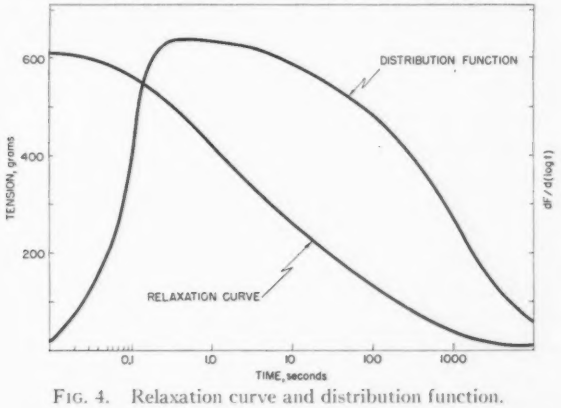


FIG. 4. Relaxation curve and distribution function.

A viscoelastic substance is frequently described by considering an array of Maxwell spring-and-dashpot elements connected in parallel (1, pp. 54–57). Mathematically, the relaxation of tension as a function of time is represented as a superposition of the forces from these elements

$$\frac{F(t)}{\gamma} = \Sigma_i E_i e^{-t/\tau_i} \qquad [1]$$

where $F(t)$ is the force, $\gamma$ the strain, $E_i$ are the moduli of the elastic springs, and $\tau_i$ are the relaxation times given by $\tau_i = \eta_i/E_i$, $\eta_i$ being the viscosities associated with the dashpots.

The properties of the relaxation curve can best be studied with the aid of a so-called distribution function which may be derived from it.

*Distribution Function*

A continuous array of spring and dashpot elements gives rise to a continuous distribution or "spectrum" of relaxation times (1, pp. 130–134) which may be characterized by a distribution function $E(\tau)$. $E(\tau)\ d\tau$ is the contribution to the total modulus of those mechanisms having relaxation times between $\tau$ and $\tau + d\tau$. The sum in equation [1] is then replaced by an integral:

$$[2] \qquad \frac{F(t)}{\gamma} = \int_0^\infty E(\tau)\, e^{-t/\tau} d\tau.$$

If $F(t)$ were known analytically, $E(\tau)$ could be found by a method of Laplace transforms (10, 11). However, several approximate methods for finding $E(\tau)$ graphically are reported in the literature (1, p. 553; 13; 14). One method (1, p. 553) which gives a good approximation is to plot $F(t)$ vs. log $t$ and then plot the negative of the instantaneous slope vs. log $\tau$. This is the method adopted in this paper and used in Fig. 4.

*Reproducibility*

It was desired to study the precision of the curves obtained and so obtain an estimate of the number of times a curve must be repeated in order to yield a desired accuracy.

Straight-grade flour of 13.1% protein, milled on a laboratory mill from spring wheat grading No. 3 Northern, was used, and experimental conditions (e.g. water content, rest period, etc.) were arbitrarily chosen so that the resulting dough would have average physical properties.

Twelve doughs were mixed with a composition of 83.2 gm. dry matter and 66.8 gm. water (55% "absorption" on a 14% moisture basis), giving a total dough weight of 150 gm. After mixing, the doughs were rounded and molded onto the dough holder and allowed a "rest period" of five minutes before being stretched on the relaxometer. The doughs were extended 1.5 cm. at a velocity of 0.74 cm./sec.

Points from the relaxograms are replotted in Fig. 5. The points were averaged at a number of time values and the mean curve drawn. Scatter is greatest in the first part of the curve and decreases approximately proportionally to the ordinate. The standard deviation for the first part of the curve is approximately 12 gm. or about 4.5%, and the standard error for the mean of the 12 curves is thus about 1.3%.

The scatter of the points on this plot is systematic rather than random in that the points that are highest at the start generally come from the same curve that produces the highest points all the way along. One may conclude that there is not as much variation in the shape of the relaxation curve as there is in its starting point. The starting point is governed by the tension built up during extension.

A similar set of curves was drawn with the relaxometer adjusted to cease extension when a tension equal to the average tension at 1.5 cm. in the above set of experiments was reached. This resulted in considerable variation in the magnitude of extension but the shape of the average curve so obtained did not differ appreciably from that shown in Fig. 5.

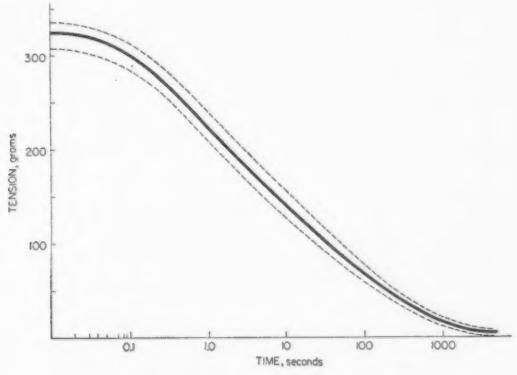


FIG. 5. Reproducibility of relaxation curves.

*Water Content*

Doughs were mixed to contain 68.7 gm. water to 100 gm. dry solids, 80.3 to 100, and 86.1 to 100 (absorptions of 45%, 55%, and 60% on 14% moisture basis) and tested on the relaxometer. The resulting curves, which are averages of at least six relaxograms each, are shown in Fig. 6. The distribution functions are shown on the same graph, where log $t$ is replaced by log $\tau$.

It may be seen that a decrease in water content increases the ordinates of the relaxation curve but does not markedly change the shape. The distri-

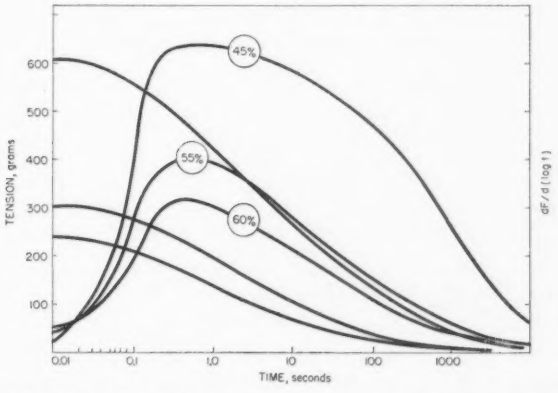


FIG. 6. Relaxation curves and distribution functions for changing water content.

bution function is therefore also increased more or less proportionately throughout its entire length.

*Rest Period*

The physical properties of a dough change markedly during resting (5). The effect of rest period (time between molding and stretching) on the relaxation curves was therefore examined. Rest period was varied from 0 to 45 min. and the resulting curves are shown in Fig. 7. There is a definite change in shape of the curves, and the distribution functions show a decrease in the ordinates corresponding to long relaxation times.

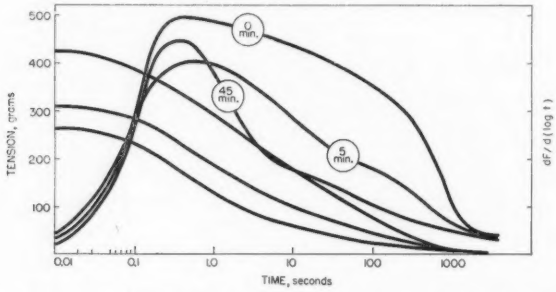


FIG. 7. Relaxation curves and distribution functions for three rest periods.

*Type of Flour*

Two additional hard spring wheat flours were studied. The flours used were: (*A*) 13.1% protein, milled from wheat grading No. 3 Northern; (*B*) 13.4% protein, milled from a mixture of wheat grading Nos. 1 and 2 Northern; and (*C*) 9.1% protein, milled from wheat grading No. 2 Northern. The curves obtained for these flours are shown in Fig. 8.

The curves and distribution functions for (*A*) and (*B*) are very similar to each other. (*B*) is about 15% higher, which is about the same as the relative difference in protein content. The low protein flour curve (*C*) is somewhat

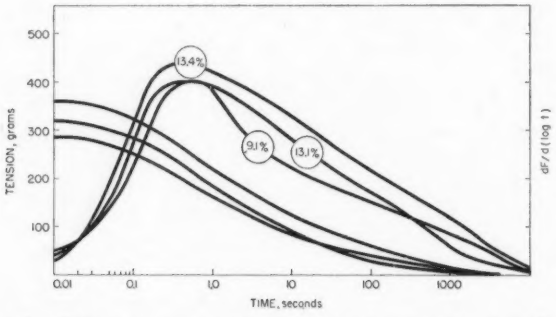


FIG. 8. Relaxation curves and distribution functions for three flours.

different in shape. Its distribution function is relatively higher in the relaxation time region of one and two seconds and somewhat lower at longer times.

## DISCUSSION

Experimenters who have studied relaxation of stress in dough have treated it from the standpoint of simple Maxwellian theory (1, pp. 54–57) of one Maxwell element with a definable elastic modulus and viscosity (7, 8, 9, 15). Examination of their data and previous work in this laboratory (6) has indicated that dough relaxation deviates markedly from Maxwellian behavior; accordingly, modulus and viscosity, and hence relaxation time, cannot be uniquely defined. More comprehensive information can be conveyed by specifying a complete curve than by considering one point on it. This type of behavior is now well known in research on rubber and other polymers. In that field, the material is represented not by a single Maxwell element but by a large number of them; one obtains a distribution of moduli and relaxation times.

Since dough behavior exhibits deviations from the simple theory, refinements are needed both in experimental methods and concepts used. The range of the distribution of relaxation times is often very great, extending over many cycles of logarithmic time. The indications were that at least four cycles would be required for dough.

Doughs contain a high percentage of water and very little is yet known about the mechanism of the effect of the water in dough. It appears, from examination of the distribution functions in Fig. 6 that the addition of water decreases the height more or less proportionately throughout the entire length. The ordinate of the distribution function is generally considered to denote the contribution to the modulus made by the mechanisms in the material having that relaxation time. It would appear from Fig. 6 that water has the effect of decreasing the contributions from all the mechanisms in the dough in a somewhat proportional manner. This might be brought about by a sort of lubricating action on the elemental components of the dough.

Rest period, on the other hand, appears to affect different portions of the distribution function in a different manner (Fig. 7). As the dough rests, the mechanisms which have relaxation times greater than one second appear to become much less prominent. It may be that when the dough is mixed many of the mechanisms are put into excited states from which they change with time to a more stable state having a relaxation time less than one second.

The curves in Fig. 8 show that the behavior of the distribution function when flours of different protein content are used appears analogous to that when rest period is changed. The high protein flours show a broad range of distribution of relaxation times; the low protein flour on the other hand predominates in shorter relaxation times.

It must be pointed out that the limit at the short time end of distribution has not been reached as it appears to be in Figs. 6, 7, and 8. The apparent limit arises from the effect of the finite extension velocities used. When the de-

formation velocity is increased the distribution appears to extend further into the short relaxation time region.

The experiments presented here as a test of the relaxometer are of an exploratory nature. Work with this improved instrument is being continued. It is felt that a study of stress relaxation of wheat dough and the distribution of relaxation times will give valuable information which it has hitherto been impossible to obtain.

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# INCREASING THE FOOD VALUE OF WHEY BY YEAST FERMENTATION

## III. PILOT PLANT STUDIES[1]

By V. E. Graham[2], D. L. Gibson[3], and W. C. Lawton[4]

ABSTRACT

When cheese whey was fermented in a vacuum pan by *Torula cremoris*, the lactose was depleted sufficiently to permit drying on a roller drum. A nonhygroscopic powder was produced containing approximately 39% protein, 19% fat, 30% ash, and 12% nitrogen-free extract, on a moisture-free basis.

## INTRODUCTION

Among the various methods used in concentrating whey to a low bulk – high nutrient feed, drying on an atmospheric drum drier is perhaps the cheapest and easiest. However, this process has several serious drawbacks. Heat from the drum causes the lactose to carmelize and form a sticky, gummy mass which rolls up on the blades. Furthermore, the dried whey powder, with its high lactose content, is very hygroscopic and unless special treatments and storage conditions are employed, the powder cakes. Several methods of drying and processing useful in the prevention of caking have been described (4–9), but most of these suggestions entail the use of expensive equipment and a high degree of technological skill.

Graham *et al.* (2) have reported that *Torula cremoris* grows readily in cheese whey, and that this organism efficiently diminishes the lactose content while simultaneously increasing the protein and riboflavin concentration. In a subsequent report (3) they described two small-scale fermenters in which the fermentation of whey by yeast had been successfully accomplished on a laboratory scale.

This paper deals with the application of their findings on a pilot plant scale. An attempt was made to grow yeasts in large quantities of whey, and to reduce the lactose content to a level which would permit drum drying without difficulty. Since this process was of primary interest to the dairy industry, it was decided to use commercial dairy equipment wherever possible.

## METHODS AND MATERIALS

The whey used in these experiments was obtained from commercial cheese operations carried out either in the University dairy or in a local factory. The whey was pasteurized at 143° F. for 30 min. and was placed under refrigerated storage until required. The yeast culture used was a *Torula cremoris* strain obtained from Iowa State College. The culture was carried in 100 ml. of sterilized whey and was transferred every 48 hr.

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*Contribution from the Department of Dairy Science, University of Saskatchewan, Saskatoon, Sask.*
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[3] *Head, Department of Dairy Science.*
[4] *Research Assistant, Department of Dairy Science.*

A flask fermenter for the preparation of inoculum was constructed from a 4-liter flask fitted with an aeration apparatus. Aeration was accomplished by forcing air through a perforated copper coil submerged below the whey surface. Forty-eight hours prior to the initiation of a trial run, 100 ml. of fresh culture were placed in this flask, 900 ml. of pasteurized whey was added, and the mixture was incubated under aeration for 24 hr. At the end of this time, 3 liters of pasteurized whey was added and fermentation was continued for an additional 24 hr. The 4 liters of culture were now equivalent to a 10% inoculum for a 40-liter batch (the usual amount processed at any one time).

Two types of large-scale fermenters were used. Since it was decided to use dairy equipment if its use was practical, a 16 in. vacuum pan was converted for this purpose. An airline introduced into the pan near the bottom served as a conduit for the incoming air. A second means of aeration was provided by using two submerged Berkfeld filters attached to an airline delivering 12 lb. per sq. in. It was found that up to 50 liters of whey could be efficiently aerated in this manner.

A second large fermenter was built to test a different principle of aeration. A metal barrel 22 in. in diameter and 34 in. deep was used as the container. Air entered by means of a hollow central shaft (see Fig. 1). This central

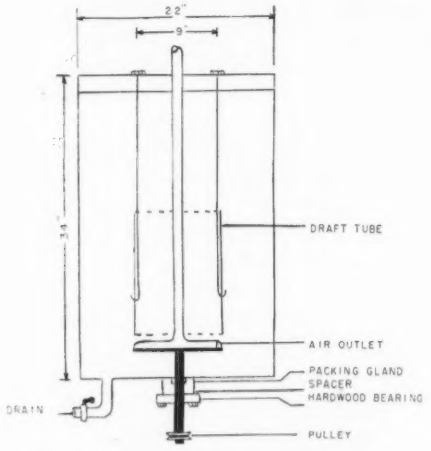


FIG. 1. Diagram of large-scale fermenter.

assembly was turned at about 900 r.p.m. by a $\frac{1}{3}$ h.p. motor. Constant temperature conditions were maintained by passing tempered water through a submerged copper coil.

The chemical analyses were performed according to methods previously outlined (3) with the exception of riboflavin assays which were carried out according to the microbiological procedure of Snell and Strong (10).

## PRESENTATION OF DATA

Most of these investigations were carried out in the vacuum-pan type of fermenter. The counts obtained in this apparatus were considerably lower than those previously reported for laboratory-scale equipment (3), but good growth occurred nevertheless. Table I illustrates the results obtained during a typical run.

TABLE I

FERMENTATION OF PASTEURIZED CHEDDAR CHEESE WHEY ON A LARGE SCALE BY *Torula cremoris*

| | 0 hr. | 24 hr. | 48 hr. |
|---|---|---|---|
| Cell population (per ml. × $10^6$) | 45.0 | 450.0 | 600.0 |
| Total solids (%) | 5.7 | 2.8 | 2.5 |
| Lactose (%) | 4.2 | 1.1 | 0.3 |
| Total solids less lactose (%) | 1.5 | 1.7 | 2.2 |
| Protein (%) | 0.89 | 1.07 | 1.06 |

Considerable difficulty was encountered in maintaining sterility of the large equipment and on several occasions microscopic examinations of the fermented whey revealed the presence of contaminants. One of these contaminants, an unidentified lactose-fermenting yeast, was isolated in pure culture and was found to increase the fermentative efficiency of *T. cremoris*. The data from one trial where both *T. cremoris* and the contaminant yeast were used are shown in Table II.

TABLE II

FERMENTATION OF PASTEURIZED CHEDDAR CHEESE WHEY ON A LARGE SCALE BY *Torula cremoris* AND UNIDENTIFIED YEAST CONTAMINANT

| | 0 hr. | 12 hr. | 24 hr. | 48 hr. |
|---|---|---|---|---|
| Cell population (per ml. × $10^6$) | 50.0 | 200.0 | 400.0 | 500.0 |
| Total solids (%) | 5.4 | 3.6 | 2.3 | 2.5 |
| Lactose (%) | 3.8 | 2.0 | 0.0 | 0.0 |
| Total solids less lactose (%) | 1.6 | 1.6 | 2.3 | 2.5 |
| Protein (%) | 0.89 | 0.89 | 1.07 | 1.2 |

It is evident from this table that the main contribution of the contaminant yeast was an acceleration of the process. Approximately the same results were obtained in 24 hr. with the mixed culture as was obtained with *T. cremoris* alone in 48 hr.

The barrel type fermenter was used on a few runs, but owing to mechanical difficulties encountered it was never possible to continue the process for a full 48 hr. period. The aeration system described for this apparatus was successful, and it appears that when the mechanical difficulties are overcome this type of fermenter might have commercial possibilities.

The whey slurry, after 48 hr. fermentation, was dried on an atmospheric drum, and the powder was analyzed. Table III lists the results of analyses of two typical runs.

Tests designed to show the hygroscopic and caking properties of these powders were negative. At the end of 10 days' exposure to normal room

TABLE III

ANALYSIS OF POWDER FROM WHEY FERMENTED BY *T. cremoris* AND MIXED CULTURE

| | Mixed culture | *T. cremoris* |
|---|---|---|
| Moisture | 10.6% | 10.9% |
| Ash | 27.0% | 27.0% |
| Protein | 40.2% | 35.6% |
| Fat | 20.1% | 17.0% |
| Crude fiber | Nil | Nil |
| N-free extract by difference | 2.1% | 9.5% |
| Riboflavin, mgm./100 gm. | 12.5 | 10.0 |
| Thiamine, mgm./100 gm. | 1.29 | 1.50 |
| Solubility* | 78% | 80% |

* *Determined according to method outlined by the American Dry Milk Institute* (*1*).

humidity there was no evidence of moisture uptake and even after water had been added artificially no tendency to cake upon air-drying could be observed after a further 20 day period. A sample of the dried yeast – whey powder retained in this laboratory for over 18 months at about 76° F. in a fiberboard carton showed no evidence of lumping after this period of time.

## DISCUSSION

Fermentation on a large scale in a vacuum-pan fermenter showed promise of becoming a feasible operation. The counts were slightly lower than in the case of the small-scale fermentations, but lactose conversion was complete. The relative inefficiency of aeration systems in the large-scale apparatus was held to be responsible for the decreased counts. This presumption was borne out by the noticeable odor of alcohol evident during the run. Both cell production and alcohol formation were shown by Graham *et al.* (3) to be closely dependent on aeration efficiency.

On the basis of chemical analysis, the whey powder manufactured during these trials was of high nutritive value. Though protein digestibility has not been ascertained, the total protein concentration is high and the powder on this basis can be ranked with linseed oil meal and cottonseed meal as a feed supplement. Furthermore, the riboflavin and thiamine content appear to be advantageous. Nevertheless, no prediction can be made in this regard without evidence from actual feeding trials.

## SUMMARY AND CONCLUSIONS

High protein powdered feed supplement has been obtained by drying whey which was fermented by *Torula cremoris*. The powder on analysis contained

approximately 39% protein, 19% fat, 30% ash, and 12% nitrogen-free extract, on a moisture-free basis.

In addition to improving the nutritive content of whey, yeast fermentation diminished the lactose content and facilitated the production of a nonhygroscopic product.

It has also been shown that fermentation of whey by yeast can be carried out on a pilot plant scale with ordinary dairy equipment. This process provides a means of whey disposal which can financially benefit cheese plant operators.

## ACKNOWLEDGMENT

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# BINDARINE AS AN ADDITION AGENT IN THE DEPOSITION OF COPPER[1]

By W. Gauvin and C. A. Winkler

ABSTRACT

It has been shown that the lignosulphonate content of bindarine is responsible for the effectiveness of bindarine as an addition agent in copper deposition. Bindarine appears to be adsorbed to only a small extent on a copper cathode. The presence of bindarine in the electrolyte increases both the hardness of the deposited copper, and the cathode polarization, but these effects are smaller than when gelatin is the addition agent.

## INTRODUCTION

Bindarine, a by-product of the sulphite pulp industry, has been used extensively as an addition agent in the electrodeposition of copper (12, 13) and other metals (2, 10), but surprisingly little has been published concerning the composition and behavior of this addition agent. It has been reported to have little influence on either the cathode or anode polarization in acid copper sulphate solutions, while the increase in polarization observed in the presence of glue and bindarine was ascribed to the glue (10). The effect of bindarine on the reflectivity of copper electrodeposits has also been studied (7). The present paper summarizes additional experimental information about the action of bindarine as an addition agent in the electrodeposition of copper for comparison with the results previously obtained with gelatin (4–6).

## EXPERIMENTAL AND RESULTS

Two samples of bindarine were used in the study. Although they were taken from production six months apart, the two samples were essentially identical in physical appearance (light yellow, free-flowing powder), in amounts of material volatile at 100° C. (7.8% and 7.9%), in ash contents (9.7% and 9.8%), and in their behavior as an addition agent. The material was very soluble in water, forming dark brown solutions, colloidal in nature, with a pH of 6.2 at a 30% concentration. The sign of the charge on the colloidal particle was determined electrophoretically, and found to be negative, unlike gelatin, which carries a positive charge. Analysis of the inorganic content, on a dry basis, gave the following:

| | | | |
|---|---|---|---|
| Calcium (as CaO) | 4.6 % | Silica | 0.014 |
| Magnesium (as MgO) | 1.31% | Total sulphur | 5.37 |
| Iron | 0.017 | Alkali metals | 0.20 |
| Alumina | 0.47 | | |

A Tingle test (14) showed that a lignosulphonate constituted a major portion of the commercial product. In an effort to determine the nature of the component of bindarine with significant properties as an addition agent,

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*Contribution from the Physical Chemistry laboratory, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.*

isolation of pure calcium lignosulphonate, without separation of the alpha and beta forms, was accomplished by Tomlinson's method (15) except that the lignosulphonate was precipitated with basic lead acetate, rather than salted out with calcium chloride. Extensive dialysis was found necessary to purify the salt completely. Methoxyl determinations on the commercial product and on the purified calcium lignosulphonate gave values of 6.34% and 11.1% respectively (cf. 11.2% and 11.35% reported by Tomlinson for alpha I and alpha II fractions of calcium lignosulphonate respectively; apparently the lignosulphonate in bindarine is the alpha form). From these relative methoxyl values, commercial bindarine presumably contains about 57% calcium lignosulphonate.

The behavior of bindarine as an addition agent was studied in an electrolyte that generally contained 125 gm. per liter $CuSO_4 \cdot 5H_2O$ (reagent grade) and 150 gm. per liter sulphuric acid (reagent grade). The anodes and cathodes, cut from rolled 18 gauge sheet, were 1 in. wide, $2\frac{1}{2}$ in. long, and submerged to a depth of $1\frac{1}{2}$ in. Before use, they were cleaned as outlined in previous papers (4–6).

Comparative experiments with bindarine as received, with the residue that remained after removal of the calcium lignosulphonate and with the extracted lignosulphonate, revealed immediately that the only component of bindarine with significant addition agent properties was calcium lignosulphonate. The deposit obtained from electrolyte containing some of the residue was identical in appearance under the microscope, and in reflectivity, with a deposit from electrolyte containing no added substance. On the other hand, addition of bindarine or of the extracted lignosulphonate gave considerably finer deposits with higher reflectivities.

Cathodes which had been dipped into solutions of gelatin, and then thoroughly rinsed, were found to yield deposits characteristic of the presence of that addition agent, as pointed out by Jacquet (9). On the other hand, deposits obtained on cathodes that had been dipped into bindarine solutions and then rinsed appeared to be identical with deposits on cathodes that had not been so treated. It may be inferred that adsorption of bindarine is relatively slight compared with that of gelatin.

The small adsorption of bindarine was further indicated by determinations of "excess weights" in the deposits obtained in the presence of this addition agent. ("Excess weight" represents the difference in weight between a deposit obtained from electrolyte containing addition agent and a deposit obtained under the same conditions from a solution containing no addition agent.) The experiments were made with considerable accuracy by simultaneous deposition on cathodes suitably suspended from the beam of an analytical balance. One cathode was immersed in electrolyte containing bindarine, the other in electrolyte to which no bindarine had been added. The cells were connected in series, and deposition allowed to occur with a current density of 2 amp./dm.$^2$ The relative "excess weights" determined for gelatin and bindarine were:

| Gelatin (mgm./l.) | Excess wt. (mgm.) | Bindarine (mgm./l.) | Excess wt. (mgm.) |
|---|---|---|---|
| 100 | 12.3 | 10 | 1.2 |
| 500 | 35.0 | 20 | 2.7 |
| 750 | 42.7 | 50 | 3.7 |
| 1000 | 44.4 | 100 | 3.7 |
| 5000 | 59.8 | 200 | 4.0 |
| | | 500 | 3.9 |

Experiments with concentrations of bindarine greater than 500 mgm. per liter were not possible, owing to its precipitation, but the much greater excess weight with gelatin is evident from the results obtained.

Combustion of finely divided samples of various deposits obtained in the presence of bindarine showed the presence of carbon in the deposit, and gave strong indication also of the presence of sulphur.

Comparative hardness tests (Rockwell, H scale, $\frac{1}{8}$ in. penetrator, 60 kgm. load) on deposits laid down in the presence of bindarine and gelatin showed that bindarine caused considerably less hardening of the deposit than gelatin, as indicated by the results shown in Fig. 1. Each value shown on the graph

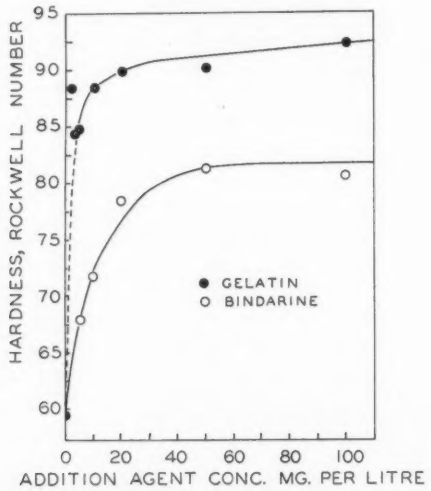


FIG. 1. Effect of gelatin and bindarine on hardness of deposit.
Temp., 25° C.
Apparent current density, 2 amp./dm.²
Copper sulphate conc., 125 gm. per liter.
Sulphuric acid conc., 150 gm. per liter.

is the average of seven determinations made at different distances (0.6 to 6 cm.) from the bottom of the cathode (1 in. × $2\frac{1}{2}$ in. long, submerged to a depth of $1\frac{1}{2}$ in.). Deposition was obtained on a single cathode between two anodes and was thick enough to overcome the influence of the base metal (11).

Extensive experiments failed to reveal any significant change in the behavior of bindarine as an addition agent as the stock solution was aged up to three weeks. There was also no indication whatever that its effectiveness altered during deposition for periods in excess of the time required to attain a steady-state polarization.

Attainment of a steady-state polarization in the presence of bindarine required some 10–15 min. longer periods of electrolysis on the standard surface (4–6) than when gelatin was the addition agent. With bindarine in the electrolyte, the steady-state polarization changed with current density, with acidity of the electrolyte, and with temperature, in the manner shown in Fig. 2.

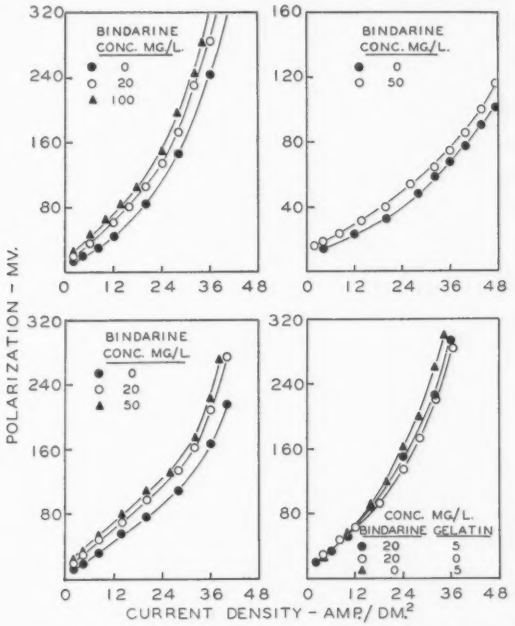


FIG. 2. Effect of bindarine on cathode polarization at various current densities. Copper sulphate, 125 gm. per liter.

Top, left: temp., 26° C.
sulphuric acid, 150 gm. per liter.
Top, right: temp., 52° C.
sulphuric acid, 150 gm. per liter.
Bottom, left: temp., 26° C.
sulphuric acid, 50 gm. per liter.
Bottom, right: temp., 26° C.
sulphuric acid, 150 gm. per liter.

It might be noted that the increase in polarization caused by bindarine was small compared with that caused by gelatin, and that the increase did not continue above 100 mgm. per liter of bindarine. An earlier report (10) of no increase in polarization is readily understood when it is realized that a concentration of 2 mgm. per liter of bindarine was used.

The acidity of the electrolyte had little effect on the increase in polarization due to bindarine, whereas with gelatin a decrease in acidity caused the polar-

ization increase to become less pronounced, especially at higher current densities. With either addition agent, an increase of temperature brought about a decrease in polarization.

The polarization resulting from a mixture of bindarine and gelatin (Fig. 2) was intermediate between that caused by either addition agent alone when present in the same amounts as in the mixture. Anode polarizations were practically unaffected by bindarine, or by bindarine–gelatin mixtures.

## DISCUSSION

As an adjunct to gelatin (glue) in refinery operations, bindarine has the advantages that it reduces the polarization and hardness of the deposit below the values observed when gelatin alone is used as the addition agent. However, bindarine does increase the polarization somewhat over that observed in its absence. This relatively small increase, compared with that brought about by gelatin, is readily explained if it is assumed that bindarine is adsorbed to a much smaller extent than gelatin, as a result of which it is much less effective in covering the active centers on the cathode, hence in increasing the true current density.

As in previous studies (5) the polarization curves in the range of current densities used conform to a general pattern, represented by the relation

$$P = K_1 + K_2(\ln D)^n$$

where $K_1$ and $K_2$ and $n$ are constants for a given curve and $D$ is the current density. The general behavior is not altered by the presence of bindarine.

It might be argued that the decreased adsorption of bindarine relative to gelatin, indicated by the smaller polarization and excess weight values, are due to the negative charge on the colloidal particle of calcium lignosulphonate, causing it to move towards the anode. This argument does not seem valid, however, since large increases in the bindarine concentration above 200 mgm. per liter do not result in corresponding increases in excess weights. This limiting adsorption is paralleled, both with bindarine and gelatin, by the limiting values for polarization and hardness curves.

Although bindarine clearly owes its addition agent effect to the presence of calcium lignosulphonate, this material as such might not be the one actually adsorbed on the cathode. Indeed, it would appear that the lignosulphonate does not function directly, since no addition agent effect was observed on a cathode that had been dipped into an aqueous solution of bindarine and subsequently used in an electrolyte containing no bindarine.

The possibility that lignosulphonic acid, precipitated in the more acid cathode film, is the addition agent also seems unlikely, since an increase in acidity of the electrolyte has not been found to alter the effectiveness of bindarine to any large extent. Also, it is doubtful that the film acidity (1) is sufficiently in excess of that in the body of the solution to bring about the precipitation. Moreover, bindarine has been shown to have beneficial effects in alkaline electrolytes (2).

Oxidation at the anode, followed by adsorption of the oxidation product at the cathode also seems an unlikely mode of action, since no appreciable time lag in the addition agent effect of bindarine was observed, nor was there any measurable change in the anode polarization.

Reduction of calcium lignosulphonate at the cathode, followed by adsorption of the products, would appear to provide the most satisfactory explanation of the behavior of bindarine as an addition agent. Aromatic sulphonic acids are electrolytically reduced to mercaptans (3) which, being weakly acid, may be either precipitated as an insoluble material or form an insoluble copper mercaptide in the more acid cathode film. Moreover, both aromatic and aryl sulphonates are powerful commercial acid pickling inhibitors (16) and are widely used as brighteners in bright nickel deposition (8). It is probable that, following reduction, adsorption of the insoluble mercaptan (or mercaptide) takes place through the polar sulphur group. The nonpolar hydrocarbon "tail" of the active material may subsequently be strongly reduced and the ring destroyed, leaving only traces of sulphur in the deposit (cf. bright nickel deposits (8)) or it may be partially destroyed and leave traces of both sulphur and carbon, as in the copper deposits obtained in the presence of bindarine.

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